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Improving the performance of factor VIII inhibitor tests in hemophilia A

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In this issue, Montalvão and colleagues [1] present important information on the value of heat treatment of specimens for inhibitor measurement in hemophilia A patients undergoing immune tolerance induction therapy (ITI). Inhibitors, antibodies directed against infused treatment products, are the most significant complication of hemophilia therapy today, occurring in up to 30% of severe patients during their initial exposures to factor VIII (FVIII) replacement. Risk of inhibitor development persists through the lifespan, with a second peak of inhibitors occurring later in life [2], and patients with all severities of hemophilia A develop inhibitors [3]. Annual testing is recommended for all patients, with more frequent monitoring for those at highest risk [4]. In addition to clinical use of the inhibitor test in detection of new inhibitors, selection of therapy, and monitoring for successful inhibitor eradication, this test is also the key endpoint measurement for evaluation of product safety, assessment of population trends, and studies of inhibitor risk factors. We have previously introduced modifications to traditional inhibitor tests to improve their usefulness for these purposes by minimizing false negative and false positive results [5,6].

The first standardized method for measuring hemophilic inhibitors, the Bethesda assay, was described in 1975 [7], with reagent modifications to improve its performance added in 1995 [8], producing what has come to be called the Nijmegen–Bethesda assay. In the past 20 years, however, the usefulness of these assays has been compromised by changes in hemophilia treatment. Patients receiving prophylactic therapy, by design, have factor present at all times and must refrain from treating to “wash out” their factor prior to undergoing traditional inhibitor tests. Presence of factor in the test specimen may result in failure to detect low titer inhibitors and produce a false negative result [5]. Patients undergoing ITI present similar problems due to frequent FVIII infusions.

Allain and Frommel [9] demonstrated that heating of inhibitor specimens to 56° for 30 min destroyed the FVIII present without affecting the antibody. Others have suggested use of this method, without documenting its effectiveness [10–12]. We provided data that the

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heating step removed both FVIII activity and FVIII antigen from plasma and showed that transition from a negative to a positive test result occurred occasionally in specimens from previously inhibitor-negative patients and frequently in those with a history of inhibitor [5]. Recently, Batty and colleagues have seen a more significant effect in patients with acquired hemophilia, in whom the detection rate for inhibitors rose from 5% to 51% when pre-analytical heat treatment was used [13]. Montalvão et al. [1] have now shown that this method also improves detection of inhibitors during ITI. In 3 of their 5 patients on ITI, negative specimens became positive after heating at some point in their monitoring, and the mean inhibitor titer for 68 ITI specimens increased 3-fold after heating. They noted that these changes could affect the prescribed course, and thus the success, of therapy.

A heating step of 56 °C for 30 min, as described by Allain and Frommel [9], was used in Montalvão et al. [1] and our studies [5,6]; others have used regimens with temperatures up to 58 °C and times as long as 90 min [12,13]. The more rigorous heating step may result in loss of FVIII antibodies measured by ELISA in some specimens [13] and cannot be recommended. Since the shorter time and lower temperature results in FVIII destruction and is known to preserve antibodies, it is preferable for this purpose.

Heat treatment of specimens has already been adopted by some laboratories to allow inhibitor testing without withholding factor infusions, eliminating one barrier to routine screening. Another barrier, however, is the high false positive rate of the assay and the risk of over-diagnosis of inhibitors. Proficiency testing internationally has documented coefficients of variation as high as 50% between laboratories and false positive rates up to 32% [14]. This methodologic variation is due primarily to differences in methods and reagents among laboratories. Additional sources of variability in clinical testing include the presence in patient plasma of substances such as heparin or non-specific inhibitors that interfere in clot-based inhibitor assays. Recently, up to 25% of low-titer inhibitor plasmas (<2 Nijmegen–Bethesda units) have been shown to lack detectable anti-FVIII antibodies [6,15], and such false positive inhibitors have influenced the results of clinical trials [15]. Recommendations have been made that low titer inhibitors should be repeated for confirmation and assessed for reactivity with factor VIII by chromogenic or immunologic assays before an inhibitor diagnosis is made [6,15]. Adoption of two steps, use of a standard method including heat treatment and confirmation of low positive results, would minimize both false negative and false positive results yet allow the detection of inhibitors at low titers, before they have major clinical impact and when they can be more easily eradicated. These steps have been adopted in a centralized testing program at the Centers for Disease Control and Prevention to provide national inhibitor surveillance for people with hemophilia in the United States [16].

References

1. Montalvão SAD, Tucunduva AC, Sambo ALD, De Paula EV, Medina SD, Ozelo MC. Heat treatment of samples improves the performance of the Nijmegen–Bethesda assay in hemophilia A patients undergoing immune tolerance induction. Thromb. Res. 2015
2. Hay CRM, Palmer B, Chalmers E, Liesner R, Maclean R, Rangarajan S, Williams M, Collins PW, the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO). Incidence of factor

VIII inhibitors throughout life in severe hemophilia A in theUnited Kingdom. Blood. 2011; 117:6367–6370. [PubMed: 21471523]

3. Soucie JM, Miller CH, Kelly FM, Payne AB, Creary M, Bockenstedt PL, Kempton CL, Manco-Johnson MJ, Neff AT, the Haemophilia Inhibitor Research Study Investigators. A study of prospective surveillance for inhibitors among persons with haemophilia in the United States. Haemophilia. 2014; 20:230–237. [PubMed: 24261612]
4. Collins PW, Chalmers E, Hart DP, Liesner R, Ranagarajan S, Talks K, Williams M, Hay CR. Diagnosis and treatment of factor VIII and IX inhibitors in congenital haemophilia. Br. J. Haematol (4th edition). 2013; 160:153–170. [PubMed: 23157203]
5. Miller CH, Platt SJ, Rice AS, Kelly F, Soucie JM, the Hemophilia Inhibitor Research Study Investigators. Validation of Nijmegen–Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveil-lance. J. Thromb. Haemost. 2012; 10:1055–1061. [PubMed: 22435927]
6. Miller CH, Rice AS, Boylan B, Shapiro AD, Lentz SR, Wicklund BM, Kelly FM, Soucie JM, the Hemophilia Inhibitor Research Study Investigators. Comparison of clot-based, chromogenic and fluorescence assays for measurement of factor VIII inhibitors in the U.S. Hemophilia Inhibitor Research Study. J. Thromb. Haemost. 2013; 11:1300–1309. [PubMed: 23601690]
7. Kasper C, Aledort L, Counts R, Edson JR, van Creveld EJ, Fratantoni J, Green D, Hampton J, Hilgartner M, Levine P, Lazerson J, McMillan C, Penner J, Shapiro S, Shulman NR. A more uniform measurement of factor VIII-inhibitors. Thromb. Diath. Haemorrh. 1975; 34:869–872. [PubMed: 1209552]
8. Verbruggen B, Novakova I, Wessels H, Boezeman M, van den Berg E, Mauser-Bunschoten. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb. Haemost. 1995; 73:247–251. [PubMed: 7792738]
9. Allain JP, Frommel D. Antibodies to factor VIII. I. Variations in stability of antigen-antibody complexes in hemophilia A. Blood. 1973; 42:437–444. [PubMed: 4579865]
10. Gatti L, Mannucci PM. Use of porcine factor VIII in the management of seventeen patients with factor VIII antibodies. Thromb. Haemost. 1984; 51:379–384. [PubMed: 6437005]
11. Kershaw G, Jayakodi D, Dunkley S. Laboratory identification of factor inhibitors: the perspective of a large tertiary hemophilia center. Semin. Thromb. Hemost. 2009; 35:760–768. [PubMed: 20169512]
12. Verbruggen B. Diagnosis and quantitation of factor VIII inhibitors. Haemophilia. 2010; 16:20–24. [PubMed: 19228204]
13. Batty P, Platton S, Bowles L, Pasi KJ, Hart DP. Pre-analytical heat treatment and a FVIII ELISA improve factor VIII antibody detection in acquired haemohilia A. Br. J. Haematol. 2014; 166:946–957. [PubMed: 24749887]
14. Favaloro EJ, Verbruggen B, Miller CH. Laboratory testing for factor inhibitors. Haemophilia. 2014; 20(Suppl. 4):94–98. [PubMed: 24762283]
15. Auerswald, G.; Kurnik, K.; Aledort, LM.; Chehadeh, H.; Loew-Baselli, A.; Steinitz, K.; Reininger, AJ.; the EPIC Clinical Study Group. The EPIC study: a lesson to learn. Haemophilia. 2015. (http://onlinelibrary.wiley.com/doi/10.1111/hae.12666/epdf)
16. Soucie JM, Miller CH, Kelly FM, Oakley M, Brown DL, Kucab P. A public health approach to the prevention of inhibitors in hemophilia. Am. J. Prev. Med. 2014; 47:669–673. [PubMed: 25245801]